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The Need for Nisin in Low-Acid Canned Foods for the Australian Defence Forces

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MRL Technical Report
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Abstract

The need to add nisin to low-acid, ration pack canned foods, which may be stored at high temperatures under operational conditions was examined. The trial showed that thermophilic spoilage was unlikely to occur in low-acid canned foods stored in the field in accordance with Service recommendations. It was concluded that the addition of nisin is not justified unless it is known that the canned foods are likely to be stored at temperatures above 40°C.

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Peter Board completed a BSc (Hons) degree in Chemistry at Sydney University in 1949 and joined CSIRO in 1950 to carry out research mainly on food processing and packaging. He was a member of several technical committees including the Armed Forces Food Specifications Committee during his career at CSIRO. He has also worked on World Bank projects in Kashmir and Uruguay and on FAO projects in Asia. He retired from CSIRO in 1987 and established a consulting company.

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The Need for Nisin in Low-Acid Canned Foods for the Australian Defence Forces

1. Introduction

1.1 Purpose of the Study

At the 115th Meeting of the Australian Defence Force Food Specification (ADFFS) Committee in September 1983, Mr P. Board of the CSIRO Division of Food Research warned that thermophilic spoilage may occur in low-acid canned foods stored under combat and survival conditions in tropical and desert areas, even though the products are produced according to the appropriate ADFFS. Mr Board pointed out that certain low-acid canned foods for the British Forces are required to contain added nisin, a non-clinical antibiotic, to minimise the risk of thermophilic spoilage. He recommended that nisin be added to those low-acid canned foods which are produced for the Services for possible use in areas involving storage at unusually high temperatures. The Committee did not accept this recommendation, but approved a proposal for a trial to be carried out at the Materials Research Laboratory - Queensland (MRL - Queensland) and at the Materials Research Laboratory - Tasmania (MRL - Tasmania). These two establishments were formerly known as the Joint Tropical Trials Research Establishment and the Armed Forces Food Science Establishment, respectively.

1.2 Previous Studies

Board (1982) stated that typical heat sterilisation processes applied to low-acid foods for the Australian Defence Force have Fo values of about 5 minutes. Such processes give a probability of a spore of *Bacillus stearothermophilus* surviving of about 1 in 10, which means that many of the cans receiving the recommended heat treatments may contain viable thermophiles. This view is shared by Murrell (1985) who stated that heat resistant, spore-forming bacteria are not infrequently found in commercially sound canned foods.

These organisms do not cause spoilage provided that the cans are stored at temperatures below the thermophilic growth range. Murrell (1985) states further that *B. stearothermophilus* is a 'flat sour' spoilage organism of low acid (pH > 5.0) foods, producing acid but none, or very little, gas as a metabolic product. He further adds that it is a facultative anaerobe, having a growth range of about 40°C - 75°C, and producing very resistant spores.

Wheaton and Hays (1964) found that the addition of nisin prevented spoilage of canned cream style corn and chow mein which had been inoculated with thermophilic flat sour spores, and incubated at 55°C for 30 to 60 days.

Gillespy (1957), in trials with peas, and beans in tomato sauce, showed that thermophilic spoilage due to *Clostridium thermosaccharolyticum* and *B. stearothermophilus* could be successfully prevented by nisin.

Barúsley (1962) similarly showed that nisin prevented spoilage in canned food prepared from contaminated raw materials and inoculated with spores of *B. stearothermophilus*.

Smith (1969) found that the addition of nisin to canned peas successfully controlled a heavy thermophilic infection, both soon after production and during storage for a period of 2 years. He also stated that the addition of nisin to the cans enabled the manufacturer to reduce considerably the severity of the thermal process.

1.3 Scope of the Present Study

This trial was designed to:

(a) determine the incidence of thermophilic spoilage in typical low-acid canned foods during storage under tropical (or desert) field conditions at MRL - Queensland and during incubation at 50-55°C at MRL - Tasmania, and

(b) determine whether the addition of nisin to these canned foods would prevent thermophilic spoilage during field storage of materials at MRL - Queensland and during incubation at 50-55°C at MRL - Tasmania.

2. Materials

The test products were commercially manufactured ration pack cans (ADFSS, 1986) of:

Green Peas (ADFFS 7-1-11), 74 x 112.5 mm cans
Mushrooms in Butter Sauce (ADFSS 7-1-25), 74 x 61.5 mm cans
Beef & Vegetable Stew (ADFSS 5-3-12), 99 x 68.5 mm cans.

The products were not inoculated with thermophilic spores.

Nisin was added at the concentration of 100 International Units per gram (2.5 ppm pure nisin) of can contents to one half of the cans of each product; the remaining cans with no added nisin acted as controls.

The brine for the Green Peas was adjusted to pH 4 with a small quantity of 2% citric acid, prior to the addition of nisin. The commercially available form of nisin, 'Nisaplin', containing 10^6 I.U. nisin per g was used.

The canned products received the following calculated heat sterilisation processes (Fo values):

Green Peas	6 minutes
Mushrooms	37 minutes
Beef Stew	4 minutes

Rochford (Edgell-Birdseye, Bathurst, NSW, Pers. Comm., 1987) confirmed the high sterilising value of 37 minutes for the Mushrooms in Butter Sauce, because the canner had encountered thermophilic spoilage in past production. The NFPA (1982) lists a (minimum) Fo value of 33 minutes for sliced Mushrooms in Brine.

After processing, the test cans (5500 total) were packed in commercial corrugated cartons, each containing two layers of twelve cans. Except for Green Peas, the layers were separated by solid cardboard liners of 0.8 mm thickness.

3. Methods

3.1 Location of Study

Half of the cans of each treatment were sent to MRL - Tasmania and the remainder were sent to MRL - Queensland. Figure 1 shows the relative location of both establishments.

MRL - Queensland was chosen due to its relatively harsh environment, typifying situations where food storage may be required during operations.

The MRL - Queensland Cowley Beach site is located 17°41'S and 146°06'E, in a hot, wet tropical zone, with appreciably saline conditions. The average rainfall of 2900 mm falls predominantly from December to May. In contrast, the cans sent to MRL - Tasmania were stored under controlled conditions in a laboratory. The cans were transported by commercial road carriers to Army Supply, thence Army transport to Cowley Beach and MRL - Tasmania. The Green Peas were processed in Northern Tasmania, the Beef & Vegetable Stew at Wagga Wagga, NSW and the Mushrooms in Sauce at Bathurst, NSW.

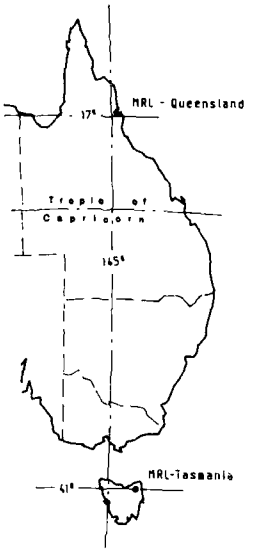

Figure 1

3.2 Storage Treatments

Four storage treatments were applied to each product as follows:

(a) 25°C (control) at MRL - Tasmania,

(b) 55°C at MRL - Tasmania,

(c) cartons stacked on wooden pallets placed on a concrete slab at MRL - Queensland, and the stacks were tightly covered by a standard olive-drab tarpaulin, and

(d) cartons stacked as in (c) at MRL - Queensland, but covered by a standard olive-drab tarpaulin erected in the manner of a tent.

As shown in Photo 1 and Figure 2, the cartons of each product were stacked three or four cartons high in double rows (numbered I and II), under two types of canvas cover. Six stacks were tightly covered by a standard olive drab tarpaulin (termed 'Close Wrap') and a further six stacks were covered by a standard tarpaulin erected in the manner of a tent (termed 'Tent Cover') as described in RAASC (1971). The cans in the field storage (Treatments c & d) remained on trial for 23 weeks, commencing in December. The cans sent to MRL - Tasmania were incubated either at 25°C for eight weeks or at 55°C for two weeks.

In summary the treatments contained three products, with and without added nisin, under four conditions of storage; a total of twenty-four lots.

Photo 1 *Cartons of cans, stacked in rows under two types of tarpaulin cover.*

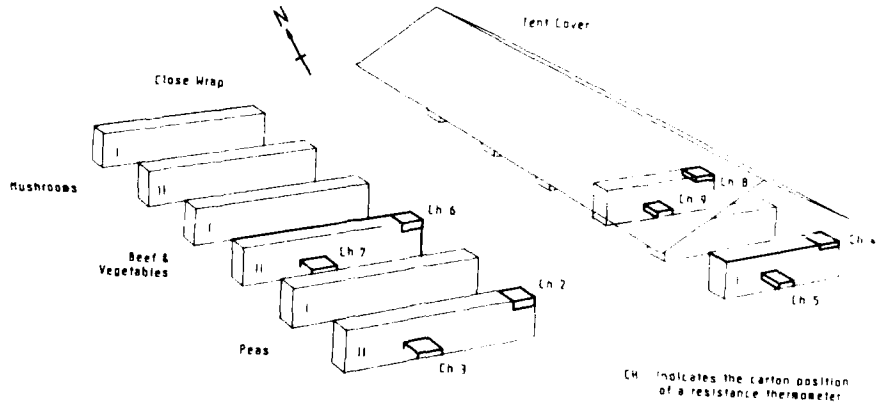

Figure 2 *Diagram showing the position of cartons in the stacks.*

3.3 Temperature and Humidity Recording

At MRL - Queensland the surface temperature of selected cans (Figure 1) was recorded hourly using resistance temperature detectors linked to a M200L Microdata Cassette Data Logger. Relative humidity and ambient temperature readings were recorded hourly at a meteorological station adjacent to the test site.

3.4 Sampling

At MRL - Tasmania, after the cans had completed their storage regimes, each can was aseptically sampled for microbiological examination of contents.

Immediately following aseptic sampling, the pH of the contents of each can was measured, using a Radiometer pH Meter Model 28 and glass electrode.

All can samples with pH of 0.5 or more pH units below that of the same nisin free product stored at 25°C (control), were examined for evidence of microbiological growth. In addition, a representative number of can samples, whose contents differed by less than 0.5 pH units from their 25°C control, were similarly examined for microbiological growth.

3.5 Microbiological Examination

Heat fixed smears stained with crystal violet were examined microscopically for the presence of micro-organisms. Samples were also cultured in Cooked Meat Medium (Oxoid), Dextrose Tryptone Broth (Oxoid) and Peptone Yeast Extract (Skerman, 1973), at 55°C. Where visual observation of the cultures indicated growth, microscopic examinations were made, and confirmatory subculturing was done using Dextrose Tryptone Agar (Oxoid) and Plate Count Agar (Oxoid), incubating at 55°C.

Tentative identification of the spoilage organism was made, using the method outline in Bergey's Manual (Buchanan and Gibbons, 1975).

The pH data of the cans were compiled and related to the results from both microbiological examination and recorded storage temperatures.

4. Results and Discussion

4.1 pH and Microbiological Examination

The means of the pH measurements for each product are summarised in Tables 1, 2 and 3. The Tables also list minima, maxima and standard deviations.

Table 1 pH of Green Peas after Storage

Treatment	Mean	Min	Max	Std. Dev.
25°C	6.10	5.80	6.30	0.10
25°C + nisin	6.03	5.85	6.30	0.12
55°C	5.17	3.87*	5.56	0.34
55°C + nisin	5.50	5.36	5.60	0.04
Tent Cover	5.86	5.78	5.98	0.04
Tent Cover + nisin	5.84	5.78	5.89	0.02
Close Wrap	5.79	5.61	5.87	0.04
Close Wrap + nisin	5.84	5.78	5.91	0.02

Total number of cans tested: 2016

* Outlier values occurred in two cans (pH 3.87 and 4.05). The remaining samples stored at 55°C had pH values within the range 4.60 to 5.56.

Table 2 pH of Mushrooms in Sauce after Storage

Treatment	Mean	Min	Max	Std. Dev.
25°C	6.34	6.25	6.45	0.04
25°C + nisin	6.55	6.45	6.65	0.05
55°C	6.44	6.40	6.50	0.03
55°C + nisin	6.63	6.55	6.70	0.03
Tent Cover	6.41	6.36	6.45	0.02
Tent Cover + nisin	6.63	6.59	6.69	0.02
Close Wrap	6.40	6.35	6.44	0.02
Close Wrap + nisin	6.62	6.52	6.71	0.03

Total number of cans tested: 1392

Table 3 pH of Beef & Vegetable Stew after Storage

Treatment	Mean	Min	Max	Std. Dev.
25°C	5.64	5.50	5.75	0.05
25°C + nisin	5.67	5.60	5.80	0.04
55°C	5.55	5.41	5.65	0.04
55°C + nisin	5.59	5.35	5.74	0.06
Tent Cover	5.67	5.58	5.75	0.04
Tent Cover + nisin	5.67	5.60	5.74	0.03
Close Wrap	5.70	5.65	5.77	0.03
Close Wrap + nisin	5.67	5.57	5.77	0.04

Total number of cans tested: 2092

The most marked change in pH occurred in the Green Peas without nisin which were stored at 55°C. A pH frequency distribution of the cans from this 55°C treatment is shown in Figure 3.

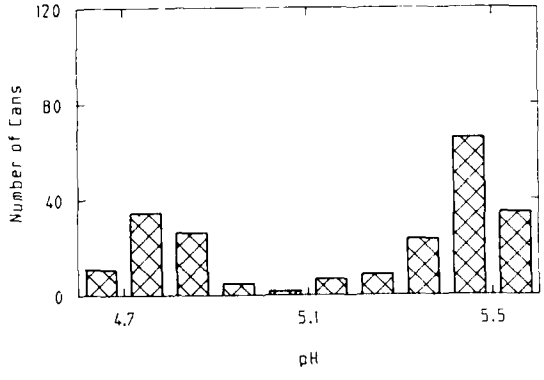

Figure 3 *pH frequency distribution for Green Peas (nisin free), incubated at 55 °C (two outlier values not included).*

Figure 3 indicates that there were two populations, one above and one below pH 5.1. Thermophilic bacteria were cultured from 82% of the samples having a pH < 5.1. Eyles (1985, p. 3.13) stated that the cultural detection of large numbers of viable spoilage organisms confirms that microbiological spoilage occurred, but that failure to detect viable organisms does not exclude microbial spoilage. He further states that autosterilisation may lead to a failure to detect viable organisms, in a food spoiled by microorganisms. Thermophiles were cultured from only one of the cans from this treatment with a pH > 5.1; that sample had a pH of 5.22. There is good evidence that about 37% of the cans of Green Peas without nisin underwent thermophilic spoilage when stored at 55°C.

In contrast, microbiological examination of the Green Peas with nisin stored at 55°C showed no evidence of thermophilic spoilage. This result is consistent with the findings of Smith (1969), where the addition of nisin to canned peas controlled a heavy thermophilic infection, both initially and during storage for 2 years. The frequency distribution (Figure 4) for treatment 55°C + nisin, shows a much narrower pH spread consistent with absence of thermophilic growth.

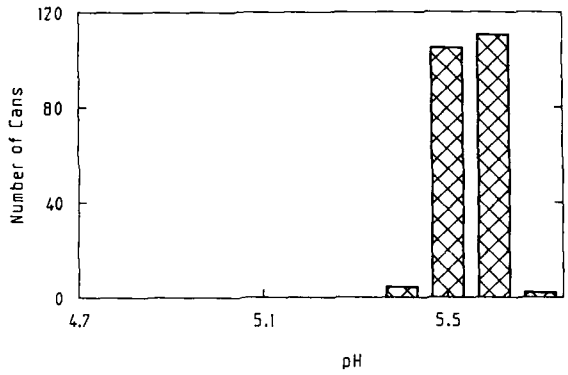

Figure 4 pH frequency distribution of Green Peas + nisin stored at 55 ℃

Apart from the spoiled samples from Green Peas without nisin, there is no evidence of microbial spoilage in any of the other cans stored at MRL - Queensland or MRL - Tasmania.

Characterisation, using the method outlined in Bergey (Buchanan and Gibbons, 1975) showed the spoilage organism to have the temperature growth characteristics of *B. stearothermophilus*.

4.2 Temperatures During Field Storage

Figure 5 depicts the percentage of storage time of 23 weeks that selected cans of Green Peas were at various temperatures. Measurement locations are shown in Figure 2. Only channel 2 (top row of cartons, under Close Wrap) recorded temperatures greater than 40°C. These temperatures persisted for 4.9% of the storage time, i.e. the equivalent of almost 8 days continuous incubation.

Figure 6 depicts the temperature of the selected cans of Green Peas and of the atmosphere on the day of maximum recorded temperature.

Channel 2 recorded the maximum temperature of 46.5°C on a day when the maximum ambient temperature was 41.7°C. A 2°C higher maximum temperature was recorded for Beef & Vegetable Stew (Channel 6), but as reported earlier there was no evidence of thermophilic spoilage in that product.

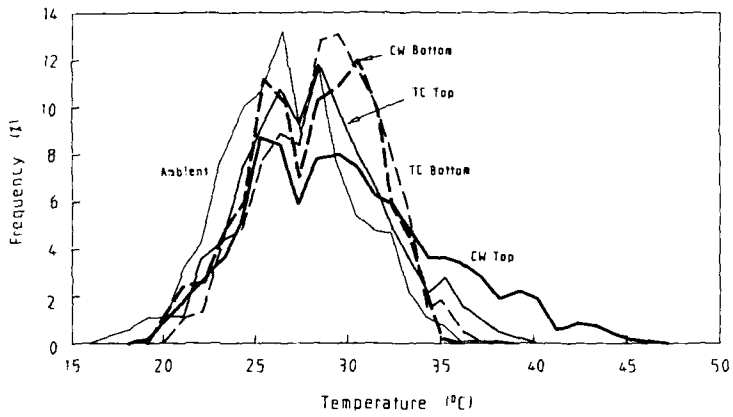

Figure 5 *Percentage of storage time (23 weeks) that selected cans of Green Peas were at various temperatures.*

Although at least some cans of Green Peas under Close Wrap were subject to a time period equivalent to 8 days incubation, in the temperature range 40°C - 46.5°C, no spoilage of these cans was detected by pH measurement or microbiological examination. This is consistent with the results of Peters (Edgell-Birdseye, Sydney, Pers. Comm. 1986), who found that *B. stearothermophilus* showed little growth at temperatures lower than 48°C.

The data in Table 1 for peas reveal a decrease in pH corresponding to increased storage temperatures and independent of microbial spoilage. The other two products did not show this pH variation with temperature. A constant difference of 0.2 pH units, between Mushrooms in Sauce with and without nisin was noted but cannot be explained; the treatments (with and without nisin) were applied to a single batch of product, but the two batches of cans were retorted separately, so the pH difference may indicate a processing variable.

As can be seen from the graphs (figures 5 and 6), temperatures are higher under Close Wrap than under Tent Cover. Maximum temperatures occurred around 1600-1900 hours, so that there is appreciable thermal inertia in the stacks. The lowest can temperatures recorded were 18°C-20°C.

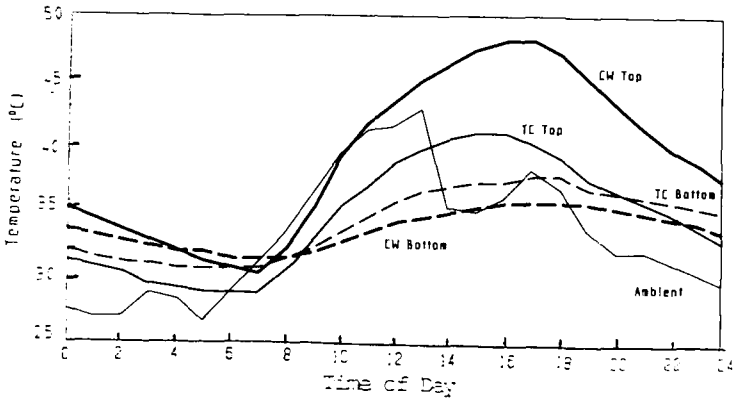

Figure 6 *Temperature of selected cans of Green Peas and of the atmosphere on the day of maximum ambient temperature.*

Figure 7 shows the sites of representative Bureau of Meteorology weather recording stations located in northern Australia and Papua-New Guinea. The suffix "+" is used to identify those sites where, for the hottest month(s), the mean maximum daily temperature for that month exceeds 40°C.

From the results of this trial, canned food temperatures at these sites would predictably exceed 47°C in close wrapped (or unprotected) cartons, with the consequent potentiality for thermophilic spoilage.

This would be of consequence if, as stated by Dibb (1986, p. 92), 'In credible contingencies, forces would be deployed to, or would operate from northern bases and over wide areas.' These results show that either the protective storage of, or the addition of nisin to thermophile susceptible, canned rations held in any 40°C plus zones (Figure 7) might be necessary.

5. Conclusions

(a) Thermophilic spoilage occurred in about one third of the cans of Green Peas without nisin that were incubated at 55°C at MRL - Tasmania for two weeks. This spoilage was evidenced by the reduction in the pH of the contents, and confirmed by culture techniques and microscopic examination. There was no evidence of spoilage in the cans of Green Peas containing added nisin.

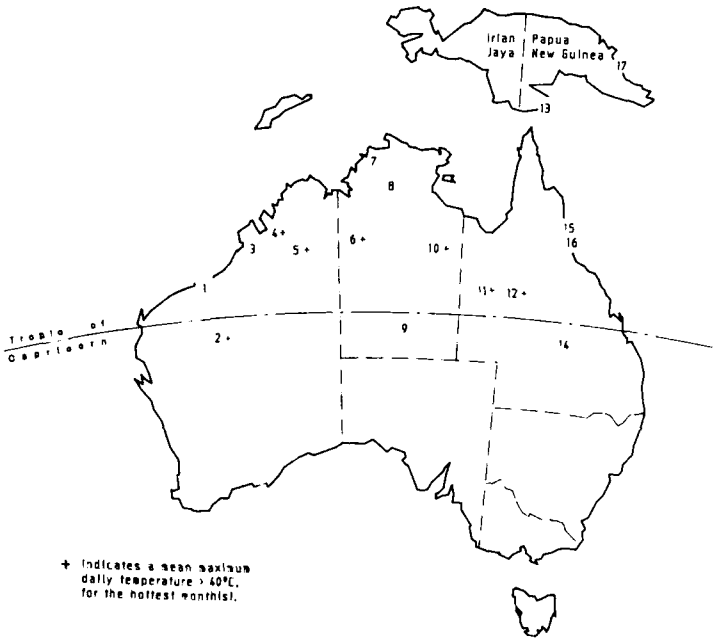

Figure 7 Representative weather recording stations, northern Australia and PNG.

(b) There was no evidence of spoilage in any other treatments, i.e. Mushrooms in Sauce and Beef & Vegetable Stew with or without nisin, incubated at 25°C and 55°C at MRL - Tasmania or stored under Close Wrap or Tent Cover at MRL - Queensland.

(c) The Green Peas stored at high temperatures showed a drop in pH in comparison to those cans stored at 25°C. This drop increased as the temperature of storage increased.

(d) The maximum temperature of the products recorded at MRL - Queensland was 48.6°C. This temperature occurred in a can of Beef & Vegetable Stew stored under Close Wrap; the can was in the top layer of the stow. The cans were at temperatures of 25°-35°C for most of the time at MRL - Queensland. The Close Wrap stacks were at temperatures > 40°C for only about 5% of the total storage period. The lowest can temperatures recorded were 18°-20°C. Temperatures were generally higher in Close Wrap stows than in cans under Tent Cover. The maximum temperatures in the stows occurred about 1600-1900 h so there was an appreciable thermal inertia. The maximum recorded ambient air temperature was 41.7°C.

6. Recommendations

(a) Nisin should not be added to the current, normally produced and distributed ADFFS canned vegetable products.

(b) It is also recommended that care be taken to store canned foods under conditions which prevent the temperature of the product exceeding 40°C. For instance, in field storage, canned foods should be stacked off the ground and under a covering which allows adequate ventilation, especially across the top of the stack. This is in accordance with RAASC (1971, Section 4-4, para 425). The covering should also protect the stacks from wind-blown rain and spray. Canned foods should not be stored in the field under close wraps.

(c) The addition of nisin to low-acid canned foods for the Armed Forces would be appropriate if it is anticipated that the cans cannot be stored at less than about 40°C and if there are doubts that the heat sterilisation process will reduce the probability of thermophiles surviving in the product to an acceptable degree.

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ABSTRACT

The need to add nisin to low-acid, ration pack cfanned foods, which may be stored at high temperatures under operational conditions was examined. The trial showed that thermophilic spoilage was unlikely to occur in low-acid canned foods stored in the field in accordance with Service recommendations. It was concluded that the addition of nisin is not justified unless it is known that the canned foods are likely to be stored at temperatures above 40°C.